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ORIGINAL ARTICLE

Additive effects of drug transporter genetic polymorphisms on irinotecan pharmacokinetics/pharmacodynamics in Japanese cancer patients

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Abstract
Purpose Effects of genetic polymorphisms/variations of *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* in addition to "*UGT1A1*28* or **6*" on irinotecan pharmacokinetics/pharmacodynamics in Japanese cancer patients were investigated.
Methods Associations between transporter haplotypes/variations along with *UGT1A1*28* or **6* and SN-38 area under the time–concentration curve (AUC) or neutropenia were examined in irinotecan monotherapy (55 patients) and irinotecan–cisplatin-combination therapy (62 patients).
Results Higher SN-38 AUC values were observed in *ABCB1* 2677G>T (A893S) (**2* group) for both regimens. Associations of grade 3/4 neutropenia were observed with *ABCC2* −1774delG (**1A*), *ABCG2* 421C>A (Q141K) and IVS12 + 49G>T (**1B*) and *SLCO1B1* 521T>C (V174A) (**15* · *17*) in the irinotecan monotherapy, while they were

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evident only in homozygotes of *ABCB1**2, *ABCG2*#*IIB*, *SLCO1B1**15 · *17* in the cisplatin-combination therapy. With combinations of haplotypes/variations of two or more genes, neutropenia incidence increased, but their prediction power for grade 3/4 neutropenia is still unsatisfactory.

Conclusions Certain transporter genotypes additively increased irinotecan-induced neutropenia, but their clinical importance should be further elucidated.

Keywords Irinotecan · Transporter · Genetic polymorphism · Haplotype

Introduction

Irinotecan, an anticancer prodrug, is widely used for treating a broad range of carcinomas including colorectal and lung cancers. However, unexpected severe diarrhea and neutropenia are important clinical side effects from irinotecan treatment. The active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin), a topoisomerase I inhibitor, is generated by hydrolysis of the parent compound by carboxylesterases [1], and is subsequently glucuronidated by uridine diphosphate glucuronosyltransferases (UGTs), such as UGT1A1, UGT1A7, and UGT1A9, to form an inactive metabolite, SN-38 glucuronide (SN-38G) [2–4]. Irinotecan is also inactivated by CYP3A4 to produce 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin (APC) and 7-ethyl-10-(4-amino-1-piperidino)carbonyloxycamptothecin (NPC) [5]. Irinotecan and its metabolites are excreted into the bile and urine via the action of ATP-binding cassette (ABC) transporters, such as P-glycoprotein (P-gp/ABCB1), multiple resistance-associated protein 2 (MRP2/ABCC2), and breast cancer resistance protein (BCRP/ABCG2) [6]. Transport of SN-38 from the plasma into the liver is mediated by the organic anion transporting polypeptide C (OATP-C/SLCO1B1) [7]. Most of the previous pharmacogenetic studies on irinotecan have focused on *UGT1A1* polymorphisms and have shown clinical relevance of *UGT1A1*28*, a repeat polymorphism in the TATA box [−54_−39A(TA)$_6$TAA>A(TA)$_7$TAA or −40_−39insTA], to severe toxicities [8–10]. Based on these findings, in 2005, the Food and Drug Administration (FDA) of the United States approved an amendment for the label of Camptosar (irinotecan HCl) (NDA 20-571/S-024/S-027/S-028) and the clinical use of a genetic diagnostic kit for the **28* allele. In parallel with this advance in the USA, clinical relevance to severe neutropenia of *UGT1A1*6* [211G>A (G71R)], another low-activity allele detected specifically in East-Asians, as well as **28* was demonstrated in several studies on Asian patients [11–14]. Accordingly, in June 2008, the Ministry of Health, Labor and Welfare of Japan approved changes to irinotecan labels (Campto and Topotecin) by adding a caution for the risk of severe toxicities in patients either homozygous or compound heterozygous for *UGT1A1*28* and **6* (**28/*28*, **6/*6*, **28/*6*) and the clinical use of a diagnostic kit for *UGT1A1*28* and **6*. Severe toxicities, however, are found in patients without **6/*6*, **28/*28*, and **28/*6*; therefore, other factors responsible for irinotecan toxicities should be identified.

Several clinical studies have suggested polymorphisms of the drug transporter genes, such as *ABCB1*, *ABCC2*, *ABCG2*, and *SLCO1B1*, might affect irinotecan pharmacokinetics (PK)/pharmacodynamics (PD) in Caucasian and Asian patients. However, the results obtained from different ethnic populations with various irinotecan regimens are still controversial, and the genetic markers examined also differ [13, 15–26]. We previously identified a number of haplotypes/variations of transporter genes, including *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* in Japanese [12, 26–29], but their clinical significance, either alone or in combination, in irinotecan therapy has not yet been examined.

This study aimed to identify the genetic polymorphisms/variations of *ABCB1*, *ABCC2*, *ABCG2*, and *SLCO1B1* which can affect irinotecan PK/PD in Japanese cancer patients. We carefully stratified the patients considering the irinotecan regimen (irinotecan monotherapy or combination therapy with cisplatin) and *UGT1A1* genotype (*UGT1A1 *6* or **28*), and examined additive effects of transporter haplotypes/variations on the area under the time–concentration curves (AUC) of the toxic metabolite SN-38 and on the risk of severe neutropenia.

Patients and methods

Patients

The patients used in this study were the same as those described in a previous paper [12], where details on the eligibility criteria for irinotecan therapy, patient profiles, and irinotecan regimens were described. In this study, 55 patients with irinotecan monotherapy (100 mg/m^2 weekly or 150 mg/m^2 biweekly) and 62 patients with combination therapy of irinotecan (60 mg/m^2 weekly or 70 mg/m^2 biweekly) and cisplatin (60 or 80 mg/m^2, respectively) were included. This study was approved by the ethics committees of the National Cancer Center and the National Institute of Health Sciences, and written informed consent was obtained from all participants.

Analyses on genetic polymorphisms and PK/PD

Patients' data on genetic variations and haplotypes of *UGT1A1*, *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* were

previously obtained [12, 26–29]. Regarding *ABCG2*, combination haplotypes were newly defined using the previously reported haplotypes from three linkage disequilibrium (LD) blocks [28]. Patients' PK data on the area under the concentration–time curve (AUC) and toxicities were previously obtained [12].

Association analyses

Associations of transporter genotypes with AUC/dose values for irinotecan, SN-38 and SN-38G, absolute neutrophil count (ANC) nadir, and incidence of grade 3 diarrhea or grade 3/4 neutropenia were investigated. For SN-38 AUC/dose and neutropenia, the patients were stratified by the presence of *UGT1A1*6* or **28* (*UGT+*). Statistical significance (two-sided, $P < 0.1$) was determined by the Mann–Whitney (MW) test or Jonckheere–Terpstra (JT) test for AUC/dose, and by Fisher's exact test and chi-square test (for trend) for incidence of grade 3 and 4 toxicities, using Prism version 4.0 (GraphPad Prism Software Inc., San Diego, CA, USA) and StatXact version 6.0 (Cytel Inc., Cambridge, MA). Multiplicity adjustment was not applied to bivariate analysis, and contributions of the candidate genetic markers to SN-38 AUC/dose values and ANC nadir were further determined by multiple regression analysis after logarithmic transformation of the AUC/dose values and ANC nadir counts. The variables examined were age, sex, body surface area, history of smoking or drinking, performance status, serum biochemistry (GOT, ALP, creatinine) at baseline, the ANC at baseline (for neutropenia), and genetic markers including *UGT1A1*6* or **28* (*UGT+*) and the transporter haplotypes. The variables in the final models were selected by the forward and backward stepwise procedure at a significance level of 0.20 using JMP version 7.0.0 (SAS Institute Inc., Cary, NC, USA).

Results

Definition of major transporter haplotypes and their selected markers

For screening transporter gene polymorphisms affecting irinotecan PK/PD, major haplotypes and their tagging single nucleotide polymorphisms (SNPs) from *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* were selected (Table 1) according to their frequencies (more than 5%) and/or from preliminary results obtained from all patients treated with irinotecan.

For *ABCB1* block 1[26], the haplotype group *BJL*, which consists of **1B* (having −1789G>A), **1J* (having −1789G>A and −371A>G) and **1L* (having −1789G>A and −145C>G), was selected because an association of the marker SNP −1789G>A with lower expression levels of P-gp has been reported [30]. *ABCB1* block 2 **2* was originally defined as haplotypes containing three SNPs, 1236C>T, 2677G>T (A893S) and 3435C>T [31]. Since the **9* haplotype with 1236C>T, 2677G>T (A893S) without 3435C>T [16] showed the same trend for PK/PD as **2* (data not shown), the current study classified the

Table 1 List of major transporter haplotypes and their markers analyzed for Japanese cancer patients

Gene	Haplotype	Tagging SNP	Abbreviation used in this paper	Haplotype frequency	
				Monotherapy ($N = 110$)[a]	With cisplatin ($N = 124$)[a]
ABCB1	*BJL*[b] (block 1)	−1789G>A		0.182	0.210
	**2* group[c] (block 2)	2677G>T(A893S)	*B*	0.382	0.379
	**10* group[d] (block 2)	2677G>A(A893T)		0.182	0.169
	**1b* (block 3)	IVS27-182G>T		0.200	0.169
ABCC2	**1A*	−1774delG	*C*	0.373	0.371
	**1C/G*	3972C>T(I1324I)		0.218	0.266
ABCG2	*#IIB* [**1a–*2–*1b*][e]	421C>A(Q141K), IVS12 + 49G>T	*G*	0.200	0.274
	#IIIC [**1b–*3–*1c*][e]	34G>A(V12M), IVS9-30A>T		0.164	0.097
SLCO1B1	**1b*	388A>G(N130D)		0.373	0.573
	**15 · 17*	521T>C(V174A)	*S*	0.191	0.153

[a] Number of chromosome

[b] *BJL* consists of **1B* (having −1789G>A), **1J* (having −1789G>A and −371A>G) and **1L* (having −1789G>A and −145C>G) previously defined [26]

[c] **2* Group includes **2*, **9*, **12* and **14* haplotypes previously defined [26]

[d] **10* Group includes **10* and **13* haplotypes previously defined [26]

[e] Combination of *ABCG2* haplotypes of three blocks [block (−1)–block 1–block 2] previously defined [28]

haplotypes with 2677G>T (A893S), *2, *9, *12 and *14 [26], as the *2 group (*2 in this paper). Similarly, the *10 group was classified as haplotypes with 2677G>A (A893T), i.e., *10 and *13, since no differences in PK/PD parameters were observed between these haplotypes. The *4, *6, and *8 haplotypes in block 2 [16, 26] showed no significant effect in the current analysis (data not shown). The *ABCB1* block 3 *1b haplotype containing IVS27-182G>T was selected because our previous study showed it was associated with an increased renal clearance of SN-38 [16].

Based on reports showing possible functional alterations of −1774delG [32] and 3972C>T (I1324I) [18, 24], *ABCC2* haplotypes containing those variations were classified as *1A and "*1C and *1G (*1C/G)", respectively, according to our previous definition: *1A, −1774delG; *1C, −24C>T and 3972C>T; *1G, 3972C>T [27]. *ABCC2*2* [1246G>A (V417I)] and *1H [2934G>A (S978S)] [27] showed no statistically significant effects (data not shown).

The *ABCG2* combinatorial haplotypes were newly defined as combinations of haplotypes across the three blocks [block (−1)–block 1–block 2] previously reported [28]. Major combinations in 177 patients were the wild type #*1A* (frequency = 0.291), #*1IB* [containing 421C>A (Q141K) and IVS12 + 49G>T] (0.251) and #*1IIC* [containing 34G>A (V12M) and IVS9-30A>T] (0.107). Note that #*1IB* and #*1IIC* are subgroups of block 1 *2 [421C>A (Q141K)] and block 1*3* [34G>A (V12M)], respectively [28].

The *SLCO1B1* haplotypes used were the major haplotypes *1b [containing 388A>G (N130D) without 521T>C (V174A)] [33] and *15 · 17 [containing 521T>C (V174A)], the functional relevance of which has been reported [34].

Association of transporter genotypes with AUC values

Since we previously found that some PK parameters, including AUC/dose, Cmax/dose and $t_{1/2}$ for irinotecan and/or its metabolites, as well as incidence of grade 3/4 toxicities were affected by irinotecan regimen [12], the following analyses were conducted using the two groups of patients; i.e., those treated with irinotecan monotherapy (100–150 mg/m^2 for initial dosage) or by combination therapy with cisplatin (60–70 mg/m^2 for initial dose of irinotecan). Since SN-38 AUC levels were largely dependent on the *UGT1A1* genotype "*6 or *28" [12], the associations of transporter genotypes with SN-38 AUC values were analyzed within the groups stratified by the marker *UGT1A1* "*6 or *28" (*UGT+*); i.e., *UGT−/−*, *UGT+/−* and *UGT+/+*. Since the SN-38 AUC/dose level of one patient with haplotypes *ABCB1*2* [2677G>T (A893S)] and *14 [2677G>T (A893S) and 1345G>A (E448K)] showed an outlying value (indicated as "*a*" in Fig. 1), this patient was excluded from the statistical analysis. In this study, we preliminarily found that effect of each transporter genotype on irinotecan PK/PD was generally small. However, it was hypothesized that multiple transporter genotypes might act additively as described below. Accordingly, we adopted a statistical significance level of $P = 0.1$ (two-sided) to pick up candidate polymorphisms for further evaluation of their combined effects.

Figure 1 shows the association of transporter genotypes with SN-38 AUC values in the irinotecan monotherapy. In all patients (ALL), higher values of the SN-38 AUC/dose were observed in the *ABCB1*2/*2* [1.64-fold of −/−, $P = 0.095$ (MW test)] (Fig. 1b) and *ABCG2*#*IIB* [1.24-fold of −/−, $P = 0.078$ (MW test)] genotypes (Fig. 1e) and lower values were observed in the *ABCB1*1b* (block 3) [0.78-fold of −/−, $P = 0.008$ (MW test)] (Fig. 1c) genotype. In *UGT−/−* patients, an increase in SN-38 AUC/dose was observed in the *ABCB1 BJL* [1.22-fold of −/−, $P = 0.073$ (MW test)] (Fig. 1a) and *ABCG2*#*IIB* [1.21-fold of −/−, $P = 0.082$, (MW test)] genotypes (Fig. 1e). In *UGT* (+/− and +/+) patients, an increase in SN-38 AUC/dose in *SLCO1B1*15 · 17 (S)* [1.59-fold of −/−, $P = 0.036$ (MW test)] was also observed (Fig. 1f). Multiple regression analysis for the SN-38 AUC/dose (logarithm-transformed values) in the irinotecan monotherapy revealed significant associations of *ABCB1*2/*2* (coefficient = 0.212 ± 0.075, $P = 0.007$), along with *UGT+/−* (0.113 ± 0.054, $P = 0.040$) and *UGT+/+* (0.225 ± 0.088, $P = 0.014$) in the final model [$R^2 = 0.226$, Intercept = 0.281 (log 10^{-3}h m^2/L), $N = 53$].

Regarding other compounds, *ABCB1*2/*2* also showed higher irinotecan AUC/dose (1.27-fold) [66.2 (48.2–82.4) [median (25th–75th percentiles)] for *2/*2 vs. 52.2 (40.6–61.9) for −/− and *2/−; $P = 0.063$ (MW test)] and SN-38G AUC/dose (1.62-fold) [18.0 (14.6–27.7) for *2/*2 vs. 11.1 (7.7–14.2) for −/− and *2/−; $P = 0.002$ (MW test)]. Conversely, lower irinotecan AUC/dose for *ABCB1*10/*10* (0.79-fold) [54.8 (44.4–65.7) for −/− vs. 43.3 (40.6–54.1) for *10/*10; $P = 0.062$ (JT test)] was detected.

For the combination therapy with cisplatin, an increase of the SN-38 AUC/dose for *ABCB1*2/*2* (1.43-fold) in *non-UGT+/+* patients (*UGT−/−* and *UGT+/−*) ($N = 55$) [3.57 (2.72–4.19) for *2/*2 vs. 2.51 (1.99–3.28) for −/− and *2/−; $P = 0.032$ (MW test)], and a decrease for *ABCB1*1b* (0.80-fold) in *UGT−/−* patients ($N = 35$) [2.03 (1.72–2.33) for *1b/− and *1b/*1b* vs. 2.55 (2.02–3.31) for −/−; $P = 0.026$ (MW test)] were observed. Multivariate analysis, however, showed no significant contributions of these transporter haplotypes to the SN-38 AUC/dose values.

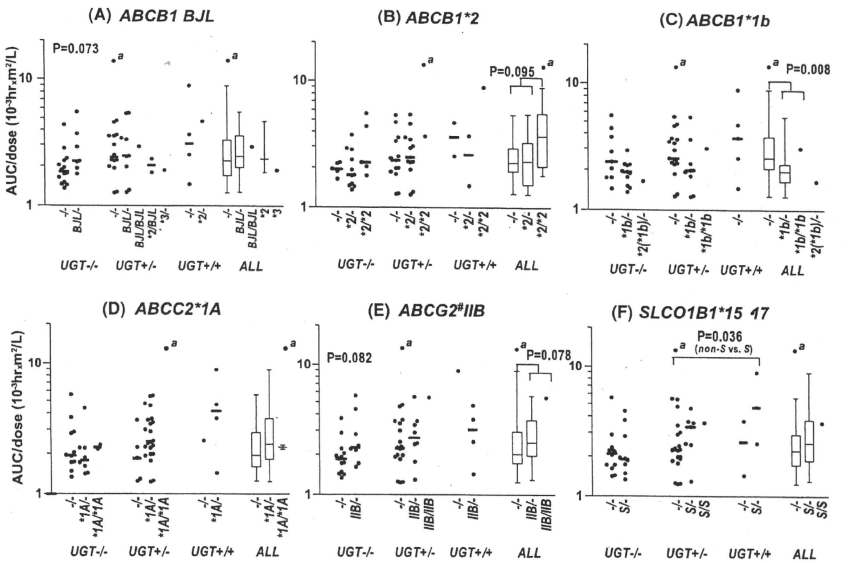

Fig. 1 Effects of transporter genotypes on SN-38 AUC/dose in irinotecan monotherapy ($N = 54$). *a* Excluded from statistical analysis. The *bars* represent the medians. *UGT+* = *UGT1A1*6* or **28*. **a** *BJL* contains −1789G>A, *2 (block 1) = 325G>A (E109K), *3 (block 1) = 304G>A (G102R); **b** **2* (block 2) contains 2677G>T (A893S); **c** **1b* (block 3) = IVS27-182G>T, **2* (block 3) = 3751G>A (V1251I); **d** **1A* contains −1774delG; **e** *IIB* contains 421C>A (Q141K) and IVS12 + 49G>T; **f** *S* = *SLCO1B1*15 · 17* containing 521T>C (V174A)

Effects of transporter genotypes on toxicities in irinotecan monotherapy

Since 80 and 100% of *UGT+/+* patients showed grade 3/4 neutropenia in the irinotecan monotherapy and combination therapy with cisplatin, respectively, neutropenia incidence was analyzed only in the *non-UGT+/+* population. Two patients were excluded from the analysis; one patient who showed an outlier SN-38 value (indicated as "*a*" in Fig. 1) and a second patient from the cisplatin-combination therapy group who discontinued irinotecan therapy.

In terms of incidence of grade 3/4 neutropenia in irinotecan monotherapy (Table 2), *ABCC2*1A*-dependent increases [0, 25.8 and 50.0% for *−/−*, **1A/−* and **1A/*1A*, respectively; $P = 0.014$ (chi-square test for trend)] were observed in *UGT* (*−/−* and *+/−*) patients. Higher incidence with *ABCG2#IIB* was also found in *UGT* (*−/−* and *+/−*) patients [9.5% for *−/−* and 35.3% for *#IIB/−* and *#IIB/#IIB*, respectively; $P = 0.049$ (Fisher's exact test)], and with *SLCO1B1*15 · 17*(*S*) in the *UGT+/−* patients [15.0, 28.6 and 100% for *−/−*, *S/−* and *S/S*, respectively; $P = 0.076$ (chi-square test for trend)].

Multiple regression analysis for the ANC nadir (logarithm-transformed values) was conducted. The final model [$R^2 = 0.466$, Intercept = 1.088 (log counts/μL), $N = 52$] revealed associations of *ABCC2*1A/*1A* (coefficient = -0.339 ± 0.088, $P = 0.0004$), *ABCG2#IIB* (-0.131 ± 0.067, $P = 0.057$) and *SLCO1B1*15 · 17* (-0.136 ± 0.066, $P = 0.046$) in addition to *UGT+/−* (-0.134 ± 0.073, $P = 0.074$) and *UGT+/+* (-0.238 ± 0.117, $P = 0.047$) and ANC at baseline (0.541 ± 0.226, $P = 0.021$), but association of *ABCB1*2/*2* was not significant (-0.158 ± 0.095, $P = 0.104$).

Although total incidence of grade 3 diarrhea was low (11%), an *ABCB1*2*-dependent increase was observed [0, 15.4 and 28.6% for *−/−*, **2/−* and **2/*2*, respectively; $P = 0.022$ (chi-square test for trend)]. Note that all patients who experienced grade 3 diarrhea had neither the *ABCC2*1C/G* nor *ABCG2#IIIC* genotypes.

Table 2 Effects of transporter genotypes on incidences of grade 3/4 neutropenia in Japanese patients treated with irinotecan monotherapy

Gene	Genotype	*UGT−/−*				*UGT+/−*				*UGT* (−/−, +/−)			
		No./total	%	*P* value		No./total	%	*P* value		No./total	%	*P* value	
				Exact[a]	Trend[b]			Exact[a]	Trend[b]			Exact[a]	Trend[b]
ABCB1	*BJL* (block 1)[c]												
	−/−	3/14	21.4	>0.1		4/15	26.7	>0.1	>0.1	7/29	24.1	>0.1	>0.1
	+/−	0/7	0.0			2/9	22.2			2/16	12.5		
	+/+					0/1	0.0			0/1	0.0		
	*2 group (block 2)												
	−/−	1/5	20.0	>0.1[d]	>0.1	5/14	35.7	>0.1[d]	>0.1	6/19	31.6	>0.1[d]	>0.1
	+/−	1/11	9.1			0/13	0.0			1/24	4.2		
	+/+	1/5	20.0			1/1	100			2/6	33.3		
	1b (block 3)[e]												
	−/−	2/9	22.2	>0.1		4/18	22.2	>0.1	>0.1	6/27	22.2	>0.1	>0.1
	+/−	0/11	0.0			2/9	22.2			2/20	10.0		
	+/+					0/1	0.0			0/1	0.0		
ABCC2	*1A*												
	−/−	0/11	0.0	>0.1	0.031	0/5	0.0	>0.1		0/16	0.0	0.022	0.014
	+/−	2/8	25.0			6/23	26.1			8/31	25.8		
	+/+	1/2	50.0							1/2	50.0		
ABCG2	#*IIB*												
	−/−	0/13	0.0	0.042		3/19	15.8	>0.1	>0.1	3/32	9.4	0.049	0.057
	+/−	3/8	37.5			3/8	37.5			6/16	37.5		
	+/+					0/1	0.0			0/1	0.0		
SLCO1B1	*15 · 17												
	−/−	2/12	16.7	>0.1		3/20	15.0	>0.1	0.076	5/32	15.6	>0.1	>0.1
	+/−	1/9	11.1			2/7	28.6			3/16	18.8		
	+/+					1/1	100			1/1	100		

[a] Fisher's exact test for (−/−) versus (+/− and +/+)

[b] Chi-square test for trend

[c] Three patients bearing *2 (block 1) or *3 (block 1) were excluded

[d] Fisher's exact test for (−/− and +/−) versus (+/+)

[e] One patient bearing *2 (block 3) was excluded

Effects on toxicities in combination therapy with cisplatin

Since only four patients (6.0%) experienced grade 3 diarrhea from the cisplatin-combination therapy, association analysis for diarrhea was not done.

Grade 3/4 neutropenia incidence was higher with *ABCB1*2* [47.1, 63.3 and 85.7% for −/−, *2/− and *2/*2, respectively; $P = 0.073$ (chi-square test for trend)] in *UGT* (−/− and +/−) patients. In *UGT*−/− patients, a higher incidence was also observed with *ABCG2*#IIB* [55.6, 83.3 and 100% for −/−, #*IIB*/− and #*IIB*/#*IIB*, respectively; $P = 0.075$ (chi-square test for trend)]. Conversely, the incidence was lower with *ABCG2*#IIIC* [71.4% for −/−, and 25% for #*IIIC*/− and #*IIIC*/#*IIIC*, respectively; $P = 0.006$ (Fisher's exact test)] in *UGT* (−/− and +/−) patients. Notably, all patients homozygous for *ABCG2*#IIB* ($N = 5$) or *SLCO1B1*15 · 17* ($N = 1$) experienced grade 3/4 neutropenia. The effect of *ABCC2*1A* on neutropenia was not consistent among the *UGT* genotypes in contrast to the results from the monotherapy. Multiple regression analysis was not applied to the neutropenia parameters in the cisplatin-combination therapy because, as described in the next section, contributions of minor variations could not be ignored.

Minor genetic variations possibly related to grade 4 neutropenia

We have detected a number of rare non-synonymous variations of the transporter genes to which statistical analysis could not be applied. Since grade 4 neutropenia

Table 3 Minor genetic variations detected in non-*UGT*+/+ patients who experienced grade 4 neutropenia

ID	Gene	Genetic variation	
		Nucleotide change (amino acid substitution)	Haplotype[a]
b1	*ABCB1*	304G>C (G102R)	*Block 1 *3*
b2(B)[b]		1804G>A (D602N)	*Block 2 *12*
b3(B)[b]		1342G>A (E448K)	*Block 2 *14*
b4		3043A>G (T1015A)	*Block 2 *16*
b5		3751G>A (V1251I)	*Block 3 *2*
c1	*ABCC2*	1177C>T (R393W)	**7*
g1	*ABCG2*	376C>T (Q126X)	*Block 1 *4*
g2		1465T>C (F489L)	*Block 2 *2*
g3		1723C>T (R575X)	*Block 2 *5*
s1(S)[c]	*SLCO1B1*	1007C>G (P336R)	
s2		311T>A (M104K)	
u1	*UGT1A1*	−3279T>G, 1941C>G	*#60-#IB* (+/+)

[a] Defined in previous papers for *ABCB1* [26], *ABCC2* [27], *ABCG2* [28] and *UGT1A1* [35]

[b] Linked with *ABCB1*2* (*B*)

[c] Linked with *SLCO1B1*15 · 17* (*S*)

occurred in *non-UGT*+/+ patients at rates of 8.0% (4/50) in the irinotecan monotherapy and 20% (11/55) in the cisplatin-combination therapy, we investigated possible contributions of these minor transporter variations and another low-activity *UGT*-haplotype, *UGT1A1#60-#IB* [35], to severe neutropenia.

Among the rare variations detected, eleven heterozygous transporter genetic variations and one *UGT1A1#60-#IB* homozygote were found in *non-UGT*+/+ patients who experienced grade 4 neutropenia (Table 3). These variations include an amino acid substitution leading to reduced in vitro activity, *ABCG2* 1465T>C (F489L) [36], and the stop codons, *ABCG2* 376C>T (Q126X) and 1723C>T (R575X) [28].

Additive effects of transporter gene haplotypes on neutropenia

Since multiple transporters are involved in irinotecan PK/PD, severity of toxicity might depend on the number and combinations of the low-activity variants, each of which does not effectively affect PD. To examine this possibility, we surveyed relationships between ANC nadirs and combinations of haplotypes associated with grade 3/4 neutropenia ($P < 0.1$) and the minor variations associated with grade 4 neutropenia (listed in the previous section); the data for selected haplotypes/variations are depicted in Fig. 2. For the combination therapy with cisplatin (Fig. 2b), homozygous *SLCO1B1*15 · 17* was included, but *ABCC2*1A* was excluded since its effect in the cisplatin-combination therapy was not consistent among the *UGT* genotypes.

In the irinotecan monotherapy, ANC nadirs in most patients with either one or more of *ABCG2#IIB*, *SLCO1B1*15 · 17* and the minor variations were lower than the median ANC nadirs of both *UGT−/−* and *UGT+/−* patients without them (None) (Fig. 2a). In particular, the effects were more evident in patients bearing two or more of the selected haplotypes/variations (including the *UGT*+). Among the patients who experienced grade 3 or 4 neutropenia, 80% of patients had two or more candidate haplotypes/variations in the *UGT* (−/− and +/−) group (Fig. 2a).

In *UGT*+/− patients with the cisplatin-combination therapy, ANC nadirs of the patients with *ABCB1*2/*2*, *ABCG2#IIB/#IIB*, *SLCO1B1*15 · 17/*15 · 17* or any minor variations, and their combinations were lower than the median values of patients without these markers (None), except for one patient with *ABCB1*2/*2* and *SLCO1B1*15 · 17* (*B/B + S/−*) (Fig. 2b). Also, in *UGT−/−* and *UGT+/−* patients, the effects were more evident in the patients with two or more of the selected haplotypes/variations. Among the patients who experienced grade 4 neutropenia, 82% of patients had two or more candidate haplotypes/variations in the *UGT* (−/− and +/−) group (Fig. 2b).

It was noted that the additive effect of *g1* [*ABCG2* 376C>T (Q126X)] was not observed in the heterozygotes (*g1/−*), but was evident in the compound heterozygotes with another *ABCG2* genetic polymorphism, *#IIB*, (*G/g1*) (Fig. 2a, b).

Regarding the combined effects of the above transporter genotypes on SN-38 AUC values, higher levels were observed in patients with the candidate haplotypes/variations of two or more genes in the monotherapy, but this trend was not always evident in the cisplatin-combination therapy patients (data not shown).

Discussion

In this study, we showed possible additive effects of transporter and *UGT1A1* genotypes on irinotecan PK and PD. Since multiple transporters are involved in irinotecan PK, it is likely that a functional alteration of one of the responsible transporters can be compensated by other transporters; thus, changes in PK/PD parameters by transporter genotypes may not always be large. However, the overall elimination rate of irinotecan or its metabolites might be altered under the conditions of simultaneously reduced activities of multiple transporters, higher irinotecan doses, or reduced UGT activity.

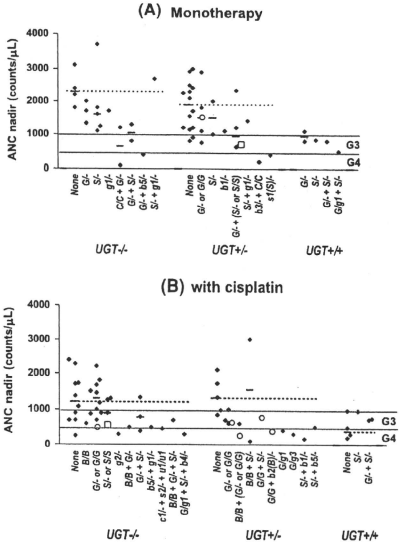

Fig. 2 Additive effects of transporter haplotypes/variations on ANC nadirs in irinotecan monotherapy (**a**) and combination therapy with cisplatin (**b**). *UGT+ = UGT1A1*6* or **28*; *B = ABCB1*2*; *C = ABCC2*1A*; *G = ABCG2#IIB* (*open circle*, *#IIB/#IIB*); *S = SLCO1B1*15 · 17* (*open square*, **15 · 17/*15 · 17*); *b1–u1* = minor variations listed in Table 3. **a** *None = non-*(*C*, *G*, *S* or *minors*), **b** *None = non-*(*B*, *G*, *S* or *minors*). The *bar* in each genotype represents the median. The *dotted lines* in each *UGT* genotype show the median values of patients without any selected transporter polymorphisms/variations (*None*). The *lines* (G3 and G4) represent the border of grade 3 and 4 neutropenia

In the irinotecan monotherapy, the increasing effect of *ABCB1*2/*2* (block 2) on SN-38 AUC/dose was evident while contributions of *ABCB1 BJL* (block 1), *ABCB1*1b* (block 3), *ABCG2#IIB* and *SLCO1B1*15 · 17* were not significant in the multivariate analysis. For neutropenia, additive effects were suggested for *ABCC2*1A/*1A*, *ABCG2#IIB*, *SLCO1B1*15 · 17*, and possibly some minor genetic variations in addition to *UGT1A1*6* or **28* (Fig. 2a). The association of *ABCB1*2* (block 2) with grade 3 diarrhea was also observed.

In the combination therapy with cisplatin, an increase in the SN-38 AUC/dose by *ABCB1*2* and for a decrease by *ABCB1*1b* were observed, but the multivariate analysis did not show their significant contributions. Regarding neutropenia, additive effects of *ABCB1*2/*2*, *ABCG2#IIB/#IIB*, and possibly, *SLCO1B1*15 · 17/*15 · 17* and some minor variations were suggested (Fig. 2b).

Thus, in both regimens, the associations of *ABCB1*2* (block 2) with higher SN-38 AUC/dose levels and toxicities (diarrhea or neutropenia), and additive effects of *ABCG2#IIB* and *SLCO1B1*15 · 17* with *UGT1A1*6* or **28* on neutropenia were observed. The current study also suggests that combination genotypes with two or more genes could have a greater effect on neutrophil count reduction than a single gene, indicating a quantitative property of multiple genetic factors affecting phenotype. These findings could partly explain a large interindividual variation in irinotecan toxicities within each *UGT* genotype.

In this study, influences of the transporter genotypes on SN-38 AUC/dose did not always correlate to an influence on neutropenia as observed in the combination therapy with cisplatin and in the case of *ABCB1*2* (block 2) in the monotherapy. Although weak negative correlations were observed between the SN-38 AUC level and ANC nadir, the SN-38 AUC values of patients who exhibited grade 3/4 neutropenia (ANC nadir < 1,000 counts/μL) were fairly diverse, especially in the combination therapy with cisplatin (Fig. 3). It is likely that the extent of toxicities depends not only on systemic exposure levels of the active metabolite for which hepatic UGT activity is a large contributor, but also on the elimination from the target cells (neutrophil progenitor cells or enterocytes) where transporter function might be more critical.

Our previous study showed the association of *ABCB1* block 2 **2* [1236C>T, 2677G>T (A893S) and 3435C>T] with lower renal clearance of irinotecan and its metabolites [16]. The current data obtained in the irinotecan monotherapy also suggest higher AUC/dose for irinotecan, SN-38G, and SN-38 with *ABCB1*2/*2*. Since a high affinity of P-gp for irinotecan is known, lower elimination rate of irinotecan could also result in higher plasma levels of its metabolites. Other studies have also suggested associations of the haplotype 1236T–2677T (corresponding to our **2* group in this study) with a reduced excretion rate of P-gp substrates [37] and SN-38 [25], and associations of the haplotype 2677T–3435T (corresponding to our **2* group in this study) with paclitaxel-induced neutropenia [38].

For *ABCC2*, *ABCC2* −1774delG, a tagging SNP of **1A*, was reported to be associated with low promoter activity and cholestatic or mixed-type hepatitis [32]. Patients with *ABCC2*1A/*1A* together with *ABCB1*2/*2* or *ABCG2#IIB* showed higher values of SN-38 AUC (Fig. 1) and neutropenia in the monotherapy (Fig. 2a), but these trends were not evident in the *UGT−/−* patients treated with cisplatin-combination therapy (data not shown). Thus, the effects of *ABCC2* might be dependent on combinations with other genetic and non-genetic factors. Conflicting clinical outcomes of *ABCC2* 3972C>T, a marker of **1C/G*, were reported to cause higher AUC of irinotecan and its

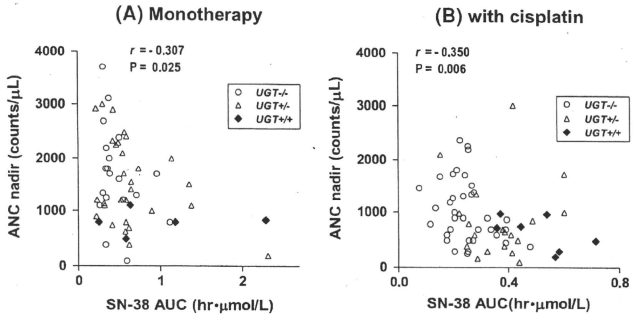

Fig. 3 Correlations between SN-38 AUC and ANC nadir in patients in irinotecan monotherapy (**a**) and combination therapy with cisplatin (**b**). *r* Spearman's rank correlation coefficient

metabolites in Caucasians treated with irinotecan monotherapy [18] and to lower the incidence of grade 3 diarrhea in Koreans treated with a combination therapy of irinotecan and cisplatin [24]. In the current study, no significant association of *ABCC2*1C/G* on PK/PD was observed in the monotherapy. Although a high incidence of grand 3/4 neutropenia was observed in patients with *ABCC2*1C/G* in the combination therapy with cisplatin, most patients also had *ABCG2#IIB* (data not shown); thus, the effect of *ABCC2*1C/G* remains obscure.

For *ABCG2*, the current study examined the association with the combinatorial haplotypes consisting of the three previously defined block haplotypes [28]. *ABCG2#IIB* contains the non-synonymous SNP 421C>A (Q141K), which was detected at higher frequencies in Asians and was reported to cause reduced expression of BCRP in vitro [36, 39–41]. In clinical studies, the association of 421C>A (Q141K) with higher plasma levels of diflomotecan was shown in Caucasians [42]. However, an association of this SNP with irinotecan PK/PD had not been shown [19, 24]. An association of 421C>A (Q141K) alone with irinotecan PK/PD was not significant in our hands (data not shown), but *#IIB* containing both 421C>A (Q141K) and IVS12 + 49G>T showed a moderate association with neutropenia. It is unclear whether the additional SNP IVS12 + 49G>T itself or another unknown linked SNP is causative for the reduced function. *ABCG2#IIIC* contains a non-synonymous SNP 34G>A (V12M) which has no influence on BCRP expression or activity in vitro [36, 39–41]. Our study showed no influence of *ABCG2#IIIC* on the SN-38 AUC/dose levels and neutropenia in the irinotecan monotherapy (data not shown), but did show a decreasing trend in grade 3/4 neutropenia in the combination therapy with cisplatin. In contrast, a report on Korean patients suggested the association of *ABCG2* 34G>A (V12M) with a higher incidence of grade 3 diarrhea in a combination therapy of irinotecan and cisplatin [24].

Among *SLCO1B1* polymorphisms, 521T>C (V174A), a tagging SNP of **15 · 17*, was demonstrated to reduce in vitro SN-38 influx [7], and clinical studies in Asians also showed its relevance to a higher SN-38 AUC and severe neutropenia in combination therapy of irinotecan with cisplatin [22–24]. Our results support these previous findings. Note that our **15 · 17* mainly consists of **17* [containing −11187G>A, 521T>C (V174A) and 388A>G (N130D)].

Taken together, the clinical data on transporter genotypes show variability among the studies. The reasons for these conflicting findings might be partly attributed to the ethnic differences in transporter genotypes and the regimens used. In addition, non-genetic factors, such as disease status and inflammation [43, 44], hepatic or renal function [45], and co-administered or pre-administered drugs, may also influence the clinical outcome.

The current study suggests combined effects of multiple haplotypes/variations on neutropenia. From clinical aspects of irinotecan therapy, the benefit of additional genotyping of transporters to predict severe toxicities should be clarified. Regarding grade 3 and 4 neutropenia, positive prediction values for two or more candidate genotypes including *UGT* (+) (Fig. 2) were 46 and 89% in the monotherapy and the cisplatin-combination therapy, respectively, which are low compared with *UGT+/+* (80 and 100%, respectively). Regarding grade 4 neutropenia, positive predictive values for these candidate genotypes were 15 and 41% in the monotherapy and the cisplatin-combination therapy, respectively, while for *UGT+/+*, they were 0 and 43%, respectively. Further studies using a

larger population size are needed to further elucidate the roles of these candidate markers.

In conclusion, the current study suggests there are additive effects for several transporter genotypes on the SN-38 AUC level and the reduction of neutrophil counts in irinotecan therapy. The clinical benefits of additional genotyping of these candidate markers should be further delineated.

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Phase II Study of Erlotinib in Japanese Patients with Advanced Non-small Cell Lung Cancer

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Abstract. *The aim of this study was to evaluate the efficacy and safety of erlotinib, an epidermal growth factor receptor tyrosine kinase inhibitor, in Japanese patients with relapsed or recurrent advanced non-small cell lung cancer (NSCLC). Patients and Methods: This was a multicentre, open-label phase II study of erlotinib (150 mg/day) in patients with stage IIIB or IV NSCLC. The primary endpoint was the objective tumour response rate. Results: Of the 46 patients, 13 were assessed to have a partial response and 9 had stable disease. The median duration of response was 449 days and time to progression was 75 days. Median overall survival (OS) was 13.5 months and the 1-year survival rate was 56.5%. The most common adverse events were dermal or gastrointestinal, and were mainly grade 2 or less. An exploratory analysis suggested a link between rash severity and OS. Conclusion: Erlotinib has promising antitumour activity and is generally well tolerated in Japanese patients with previously treated NSCLC.*

Lung cancer is the most common cancer worldwide, with almost 1.5 million new cases diagnosed every year, and it is also the leading cause of cancer-related death (1-3). Non-small cell lung cancer (NSCLC) is the most common form of lung cancer (accounting for approximately 85% of cases) and because early-stage NSCLC is often asymptomatic, close to 70% of patients present with advanced (stage IIIB or IV) disease (3). The prognosis for patients with NSCLC remains poor, with 5-year survival rates of 5-10% and median survival times of 12-15 months (3, 4).

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Treatment approaches in NSCLC vary according to the extent of the disease (5). Surgery offers the chance of a cure in early disease, and combining surgery with chemotherapy can improve outcomes (6). However, advanced NSCLC cannot be resected and is therefore generally incurable. As a result, the major treatment goals in advanced NSCLC are to delay tumour progression (thereby increasing survival), delay worsening of symptoms, and to maintain or improve quality of life. Standard first-line treatment for metastatic NSCLC is platinum-based chemotherapy with the addition of third-generation agents (*e.g.* paclitaxel, gemcitabine, vinorelbine or irinotecan) (7, 8). However, it is generally accepted that a plateau in efficacy has been reached in NSCLC for traditional chemotherapy regimens (3).

Erlotinib (Tarceva®) is a highly potent, orally active epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI). Erlotinib has proven efficacy in Japanese patients with advanced NSCLC (9), and was approved in Japan for the treatment of relapsed NSCLC in October 2007. The pivotal BR.21 study showed that erlotinib has a beneficial effect on survival in a wide range of patients with NSCLC, irrespective of biomarker status (10). However, in this trial, patients of Asian ethnicity were found to have a significantly higher response rate than other patient groups combined (18.9% *vs.* 7.5%; p=0.02). One possible explanation is that Asian patients have a higher rate of tumors with *EGFR* mutations, and are more likely to respond to EGFR-TKI therapy. Recent studies indicate that response to EGFR-TKIs may be predicted by the presence of *EGFR* gene mutations (11-13), suggesting a role for biomarker-based tailored therapy.

Dermal toxicities, such as rash, pruritis and dry skin are major treatment-related adverse events (AEs) of erlotinib. The occurrence and severity of rash has been linked to the clinical efficacy of erlotinib in patients with NSCLC (14-16), suggesting that rash may be a surrogate marker for improved response to therapy.

This paper reports the findings of a phase II study of the efficacy and safety of erlotinib in Japanese patients with relapsed or recurrent advanced NSCLC. The study also examined the possible correlation between rash and survival time in patients receiving erlotinib, and a biomarker analysis was conducted.

Patients and Methods

This multicentre, open-label phase II study recruited patients at 11 sites in Japan. The primary endpoint was the objective tumour response rate (ORR), measured in accordance with Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (17). An external confirmation of antitumour efficacy was conducted by an independent response evaluation committee. Secondary endpoints were the disease control rate (DCR), duration of response, time to progression (TTP), overall survival (OS), 1-year survival rate, quality of life (QoL) and safety.

Patients. Patients (aged 20-74 years) with histologically or cytologically documented stage IIIB or IV NSCLC that was recurrent or refractory to treatment, and who had received at least one prior chemotherapy regimen, were enrolled in the study. Eligibility criteria included: measurable lesions (by RECIST) not curable by surgery or radiotherapy; an Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 0-2, and adequate bone marrow function, hepatic function (aspartate aminotransferase [AST], alanine aminotransferase [ALT] levels ≤2.5 times and total bilirubin ≤1.5 times the upper limit of normal [ULN]), renal function (serum creatinine ≤1.5 times ULN) and pulmonary function (arterial oxygen pressure [PaO_2] ≥70 Torr). Patients had to complete their last cycle of chemotherapy at least 4 weeks prior to the study, and their last course of thoracic radiotherapy had to have been at least 12 weeks previously. Patients were excluded from the study if they had a history or complications of interstitial lung disease (ILD) (scarred radiation pneumonitis limited to the field of radiation was permitted) or current ophthalmological abnormalities (dry eye syndrome including Sjögren's syndrome, severe dry keratoconjunctivitis, keratitis). Written informed consent was obtained from all patients.

Study design and treatment. All patients received 150 mg erlotinib once daily before breakfast, until the occurrence of progressive disease (PD) or unacceptable toxicity. In the event of treatment-related toxicity, two dose reductions were permitted per patient (first reduction to 100 mg/day, second reduction to 50 mg/day), and dosing could be interrupted for up to 14 days. No dose escalations were permitted. For grade 3 or intolerable grade 2 rash or stomatitis, treatment was discontinued until improvement to grade 2 or less, and then a lower dose of erlotinib was started. For any other grade 3 treatment-related toxicities, treatment was interrupted until improvement to grade 1 or less and then the same dose was re-started. For ILD of any grade or grade 4 toxicity, treatment was permanently discontinued.

Efficacy evaluation. Tumour assessments were evaluated in accordance with RECIST (17) and were performed at baseline and every 4 weeks until week 16, then every 8 weeks thereafter. Confirmation of complete or partial responses (CR or PR) was obtained by a second assessment conducted ≥28 days after the initial assessment. Stable disease (SD) was defined as disease control (absence of progression) maintained for at least 6 weeks. An independent response evaluation committee, consisting of two oncologists and a radiologist, reviewed images of patients with CR, PR and SD. Individual survival times were calculated during the study period and at the post-study follow-up survey, and OS was defined as time from first erlotinib administration to death.

Safety evaluation. Baseline assessments included a full patient history, physical examination, standard laboratory tests, electrocardiography, chest radiography and ophthalmology tests (visual acuity test, slit-lamp examination). Vital signs and ECOG PS were monitored and blood samples were taken every week until week 8, and every 2 weeks thereafter. In addition, a radiograph to assess pulmonary toxicity was conducted weekly until week 4, and every subsequent 2 weeks, and ophthalmological tests were repeated at week 8 and at the end of the study. AEs were monitored throughout the study and graded using National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0. For ILD-like events, the data safety monitoring board (which consisted of oncologists and pneumologists) reviewed the clinical data and

Table I. *Summary of patient baseline characteristics and demographics.*

Characteristic	Number of patients (%) (n=46)
Median age (range), years	60.0 (38-74)
Gender	
Male	27 (59)
Female	19 (41)
Performance status	
0	24 (52)
1	22 (48)
Histology	
Adenocarcinoma	40 (87)
Squamous cell	4 (9)
Large cell	1 (2)
Adenosquamous carcinoma	1 (2)
Stage	
IIIB	3 (7)
IV	31 (67)
Recurrent	12 (26)
Smoking history	
Never	22 (48)
Former	21 (46)
Current	3 (7)
Median time since initial diagnosis, days (range)	280.5 (3-3452)
Prior chemotherapy regimens	
1	23 (50)
2	12 (26)
≥3	11 (24)
Prior taxane treatment	
No	14 (30)
Yes	32 (70)
Median time since last regimen, days (range)	62.0 (29-939)

images: the images were also examined by a review committee of radiologists with expertise in drug-induced pulmonary disorders.

QoL evaluation. QoL was assessed using the Functional Assessment of Cancer Therapy-Lung (FACT-L) questionnaire (version 4-A) (18). The full FACT-L questionnaire was administered at baseline and every 28 days, and the Lung Cancer Subscale (LCS), an independently validated component of FACT-L, was administered weekly during the treatment period except for the extension study period. Symptomatic improvement in LCS was defined as an increase of two or more points from baseline, sustained for at least 4 weeks and best responses were analysed for all patients with a baseline score of 24 or less (out of a possible 28).

Biomarker analysis. Tumour samples were obtained for biomarker analysis as formalin-fixed and paraffin-embedded blocks, or as thinly sliced tissue sections mounted on glass slides (at least five slides were examined). *EGFR* gene mutations were assessed at first diagnosis or surgery, when tumour specimens were available. These assessments were only carried out with separate written consent. The tumour tissue was laser microdissected at Targos Molecular Pathology GmBH (Kassel, Germany) and direct sequencing was then carried out at the Roche Centre of Medical Genomics (Basel, Switzerland) using a nested polymerase chain reaction (PCR) to amplify exons 18-21.

Table II. *Response to erlotinib (core study period).*

	Number of patients (%)
Partial response	13 (28.3)
Stable disease	9 (19.6)
Progressive disease	20 (43.5)
Not evaluable	4 (8.7)
Response rate (%) (95% CI)	28.3 (16.0-43.5)
Disease control rate (%) (95% CI)	47.8 (32.9-63.1)
Median time to progression (days)* (95% CI)	75 (56-**)

*Kaplan-Meier analysis; **not estimated.

Pharmacokinetics. The pharmacokinetic profiles of erlotinib and its *O*-desmethylated metabolite OSI-420 were analysed at baseline, and weeks 2, 4 and 8. Plasma concentrations of erlotinib and OSI-420 were measured by reverse-phase liquid chromatography-tandem mass spectrometry (LC-MS/MS) (19).

Statistical analysis. Given an expected overall response rate (ORR) of 25%, a Fisher's exact test was performed (two-sided α=5.0%). Based on 40 patients, the power to test the null hypothesis (ORR=5%) was 95.67%. In the event that the true ORR was proven to be 20%, the power to test the null hypothesis (ORR=5%) would be 83.87%. The target sample size of 45 patients was chosen on the expectation that a proportion of patients would prove to be ineligible for the study. Efficacy analyses were conducted on the full analysis set, which was produced by omitting ineligible patients. The 95% confidence intervals (CIs) for ORR, DCR, and symptom improvement were calculated using the Clopper-Pearson method. Time-to-event variables were estimated using Kaplan-Meier method. Cox proportional hazards regression analysis of OS was conducted to evaluate the effects of 11 factors related to patient characteristics and treatment history.

Results

Patient characteristics. A total of 46 patients were recruited and participated in the study period between January 2005 and January 2006 (Table I). Fifteen patients who maintained a response or SD to erlotinib at January 2006 were able to continue with treatment. Efficacy and safety were continuously assessed for these patients in an extension study until January 2008. All 46 patients were evaluable for safety and efficacy. Patients had a median age of 60 years (38-74 years) and 27 (59%) were male. Forty (87%) patients had adenocarcinoma and 22 (48%) were never smokers. Erlotinib administered in the current study was second-line treatment for half of the 46 patients recruited and the proportion of patients who were to receive erlotinib as third- or fourth (or greater) -line was similar (26% and 24%, respectively).

Efficacy. Overall, 13 patients were assessed as having a PR and nine as having SD (Table II). Objective response could not be confirmed in four patients: three patients discontinued erlotinib early after the first administration because of AST, ALT

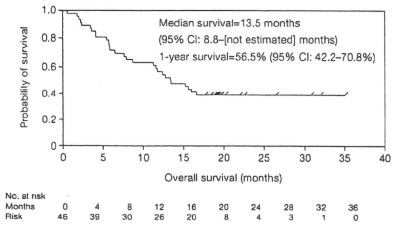

Figure 1. *Kaplan-Meier plot of overall survival (including extension study period).*

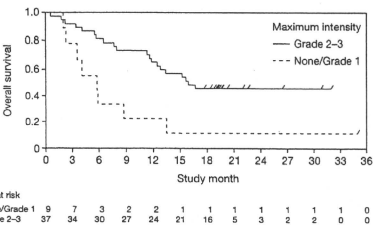

Figure 2. *Kaplan-Meier plot showing the relationship between rash and overall survival (including extension study period).*

elevation, withdrawal of informed consent or patient's refusal, and the fourth patient was not evaluable due to lack of baseline assessment for non-target lesions. The ORR was 28.3% (95% CI: 16.0-43.5%) and the DCR was 47.8% (95% CI: 32.9-63.1%). The symptom improvement rate, measured using the LCS, was 35.7% (15/42, 95% CI: 21.6-52.0%).

The median duration of response, TTP and OS were also evaluated, including data from the extension study period up to January 2008. The median duration of response was 449 days (95% CI: 295 days-[not estimated]) and TTP was 75 days (95% CI: 56-263 days). Median OS was 13.5 months (95% CI: 8.8 months-[not estimated]) and the 1-year survival rate was 56.5% (95% CI: 42.2-70.8%) (Figure 1).

A Cox regression analysis of OS showed that only gender was a significant predictor for OS (Table III).

Pharmacokinetics. Pharmacokinetic parameters were evaluated in 40 patients; however, mean trough concentration data at steady-state ($C_{ss,min}$) were available for only 36 patients as baseline sampling was not performed in 4 patients. The results showed that $C_{ss,min}$ of erlotinib did not vary significantly over time, with stable levels reached by around day 15 and maintained until day 57. The mean $C_{ss,min}$ values (±standard deviation) of erlotinib on days 15, 29 and 57 were 1085.8±660.9 ng/ml, 1001.5±727.2 ng/ml and 981.3±528.5 ng/ml, respectively (average 1063.8±657.0 ng/ml). The corresponding mean values for OSI-420 were 92.4±81.2 ng/ml, 83.6±84.5 ng/ml and 81.9±61.8 ng/ml, respectively (average 88.5±75.1 ng/ml). There was no statistically significant difference in $C_{ss,min}$ based on patient characteristics (age, gender, tumour histology or smoking status) or major AEs.

Biomarker analysis. Paraffin-embedded tissue samples were available for 15/46 patients and there was sufficient tumour tissue lysate to carry out DNA sequencing to determine *EGFR* mutation status in six of these samples. All six patients for whom *EGFR* mutation analysis was carried out had adenocarcinoma (Table IV): three were never smokers and three were former smokers. *EGFR* mutations were identified in three patients, two of whom experienced PR (both have exon 19 deletions) and one who had PD (exon 19 point mutation).

Safety. All 46 patients who received erlotinib were assessed for safety, and treatment-related AEs were observed in all patients (treatment-related AEs with ≥20% incidence are shown in Table V). The most common events were rash, experienced by 45/46 (97.8%) patients, of which 91.3% cases were grade 1 or 2, diarrhoea (31/46 [67.4%] patients, 52.2% grade 1), pruritis (30/46 [65.2%] patients, 50.0% grade 1) and dry skin (28/46 [60.9%] patients, 54.3% grade 1). All events with an incidence >30% were gastrointestinal or skin disorders.

AEs led to discontinuation of erlotinib in 4 patients. One patient (aged 60 years) developed interstitial pneumonia on day 8, and this resulted in death. A computed tomography scan showed that this patient exhibited the characteristic features of ILD, and the ILD review committee judged that the event may possibly have been related to erlotinib. The remaining three patients who discontinued erlotinib did so because of ALT elevation, ALT and AST elevation, and fever, respectively. The case of fever was later found not to be directly related to study treatment. Twenty patients (43.5%) required dose interruption. The main reasons for the dose interruptions were rash (9/46 [19.6%]), ALT elevation (5/46 [4.2%]) and AST elevation (4/46 [8.7%]). Ten patients (21.7%) had dose reduction due to AEs, mostly due to rash (6/46 [13.0%]). Furthermore, there were no intolerable clinical episodes during the extension study

Dose intensity and duration of treatment. To assess the feasibility of treatment, we evaluated compliance with treatment for patients experiencing PR and SD (22 patients). The treatment duration of patients with PR or SD was a mean of 375.4 days (median=317 [43-1066]) days. The mean and median relative dose intensity of responders and patients with SD was 88.6% and 96.9%, respectively. Among these patients, 13 did not require a dose reduction and one patient was treated with erlotinib for 1066 days without dose reduction.

Table III. *Cox regression analysis of survival (including extension study period).*

	Hazard ratio*	95% Confidence interval	*p*-Value
Univariate analysis			
Age (≥65 *vs.* ≤65 years)	0.94	0.41-2.14	0.883
Gender (female *vs.* male)	0.34	0.15-0.77	0.010
Histology (adenocarcinoma *vs.* non-adenocarcinoma)	0.28	0.11-0.76	0.012
Smoking history (never *vs.* current or former)	0.48	0.23-1.03	0.060
Performance status (0 *vs.* ≥1)	0.65	0.31-1.38	0.259
Prior regimens (≥2 *vs.* 1)	0.98	0.47-2.07	0.967
Baseline serum KL-6 (<median, 465 U/ml *vs.* ≥median)	0.79	0.37-1.69	0.540
Best response to previous chemotherapy (non-PR *vs.* PR)	0.68	0.29-1.60	0.373
Prior taxane therapy (no *vs.* yes)	0.61	0.26-1.44	0.259
Time since initial diagnosis (>12 months *vs.* ≤12 months)	0.68	0.30-1.54	0.678
Final model			
Gender (female *vs.* male)	0.34	0.15-0.77	0.010

*Left of '*vs.*' indicates reference group; KL-6, a mucinous glycoprotein expressed on type II pneumocytes; PR, partial response.

Table IV. *EGFR mutation analysis (including extension study period).*

Response	TTP (days)	Survival (days)	Gender	Histology	Smoking history	Mutation status	Exon	Type of mutation
PR	308	599+	F	Adenocarcinoma	Never	+	19	Del L747 - P753 ins S
PR	973+	973+	F	Adenocarcinoma	Never	+	19	Del L747 - P752 ins PL
SD	116	669+	F	Adenocarcinoma	Never	–	–	–
PD	28	559+	M	Adenocarcinoma	Former	–	–	–
PD	57	356	M	Adenocarcinoma	Former	+	19	I759T
PD	29	597+	M	Adenocarcinoma	Former	–	–	–

PR, Partial response; SD, stable disease; PD, progressive disease; TTP, time to progression; + censored.

Exploratory analysis of a relationship between rash and OS. An exploratory analysis suggested a link between rash severity and OS. Kaplan-Meier analysis showed an advantage in terms of survival time for patients with rash grade 2 or 3 compared with those exhibiting rash grade ≤1 (Figure 2). Patients with a maximum rash grade ≤1 had a median OS of 5.8 months compared with 16.0 months for those with rash grade 2 or 3.

Discussion

In the current study, erlotinib of 150 mg/day achieved an ORR of 28.3%, which is higher than that observed in phase II and phase III studies of erlotinib (second- or subsequent-line) in NSCLC conducted in the United States (12.3% (14); 18.9% (10) [Asian subpopulation]), but the same as that seen in a previous phase II study carried out in Japan (28.3% (9)). The characteristics of patients in this study were generally similar to those of NSCLC patients as a whole, in terms of their demographics and disease and treatment history, with the exception of a particularly high proportion of patients with adenocarcinoma (87%) and those never having smoked (48%). However, the possibility of enrolment bias on the basis of histological type cannot be ruled out, in part because enrolment coincided with some reports regarding the clinically predictive factor of EGFR-TKI therapy (20-22).

The median survival time with erlotinib was a promising 13.5 months, which is similar to that reported with erlotinib in a recent phase II study of Japanese patients with NSCLC (14.7 months) (9). One-year survival rates were the same in these two studies (56.5%), and the median TTP (75 days) was similar to that reported in previous studies of patients with advanced or recurrent NSCLC conducted in the United States and Japan (9, 10, 14). Together these data provide a convincing body of evidence supporting the efficacy of erlotinib in patients with advanced NSCLC.

Pharmacokinetic analysis showed that steady-state concentrations of erlotinib were reached after 15 days, were maintained for the 2 months of analysis, and were not affected by patient characteristics such as gender or smoking history. This supports a previous analysis where no significant differences were seen between a phase I study of Japanese patients (23) and a phase I study in Western patients (24) in terms of the pharmacokinetic profile of erlotinib. In contrast to the present study, another previous study demonstrated that current smokers had significantly less erlotinib exposure than non-smokers (25). The reasons underlying this difference are

Table V. *Major treatment-related adverse events with an incidence ≥20% (including extension study period).*

Event*	Number of patients (%)	NCI-CTC grade			
		1	2	3	4
Rash	45 (97.8)	8	34	3	0
Diarrhoea	31 (67.4)	24	6	1	0
Pruritus	30 (65.2)	23	7	0	–
Dry skin	28 (60.9)	25	3	–	–
Stomatitis	21 (45.7)	16	4	1	0
Anorexia	16 (34.8)	11	2	3	0
Paronychia	15 (32.6)	11	4	0	0
T-Bil increased	13 (28.3)	6	7	0	0
ALT increased	12 (26.1)	5	3	3	1
C-Reactive protein increased	11 (23.9)	10	1	0	0
Fatigue	11 (23.9)	8	3	0	0
Nausea	10 (21.7)	9	1	0	–

NCI-CTC, National Cancer Institute Common Toxicity Criteria; T-Bil, total bilirubin; ALT, alanine aminotransferase. *Categorised by MedDra Ver.7.1 (the Medical Dictionary for Regulatory Activities Ver.7.1).

unclear; however, it is possible that the small numbers of current smokers enrolled in our study may have been a contributing factor.

As in previous studies of erlotinib in NSCLC, the observed AEs were predominantly dermal or gastrointestinal in nature and, although they occurred at frequencies of 50% or more, they were generally classified as grade 2 or lower. Although the frequency of these AEs was higher than that seen in patients receiving erlotinib in the pivotal BR.21 US phase III study (10), the frequency of severe toxicities (grade 3 or greater rash or diarrhoea) was not. These findings did not support the magnitude of the difference seen between the BR.21 and the Japanese phase II study populations, with the exception of ethnic difference. Further studies are needed to clarify the influence of ethnic difference on the frequency and severity of erlotinib-induced toxicities.

Notably, erlotinib is not associated with the haematological toxicities that are often seen with standard chemotherapy such as docetaxel, and there is no need for co-medications or routine monitoring. The main events associated with erlotinib, rash and diarrhoea, can be effectively managed using symptomatic treatment, dose reduction and/or suspension of administration. One patient died due to ILD in this study. As similar cases of ILD-related death have been reported in previous studies, we recommend that careful screening of patients for ILD risk factors (signs of pulmonary fibrosis or interstitial pneumonia) should be carried out before prescribing erlotinib.

The favourable tolerability profile of erlotinib enabled patients to remain on treatment for long periods: the median treatment duration was more than a year, and one patient received erlotinib for 1066 days. We also found that improved OS was correlated with the severity of rash in this study, as has been noted by other investigators (26). Therefore, active management of rash may be an important consideration for prolonged survival without QoL deterioration.

Mutations in the kinase region of the *EGFR* are thought to enhance sensitivity to EGFR TKIs such as erlotinib and gefitinib. A meta-analysis of 1170 patients has shown that more than 70% of patients with *EGFR* mutations respond to TKIs, whereas only 10% of patients without *EGFR* mutations responded (27). However, the link between *EGFR* mutational status and survival is not straightforward, and there may be some other factors influencing the efficacy of EGFR-TKIs, such as *EGFR* copy number, status of other members of the EGFR family, and somatic mutations of downstream molecules such as *KRAS* (13, 28, 29). *EGFR* mutation analysis was only possible for six patients in the current study, and two out of the three patients with *EGFR* mutations experienced a (partial) response. A significant amount of work is required to determine the relationship between such biomarkers and OS in Japanese patients receiving erlotinib.

In summary, erlotinib was found to have promising antitumour activity in this phase II study of Japanese patients with previously-treated NSCLC. Erlotinib of 150 mg/day was well tolerated by most patients and the AE profile was in line with that seen in previous studies with similar patient populations. There was some evidence to suggest a correlation between the severity of rash and improved survival. *EGFR* mutation analysis was only possible for six patients and therefore definitive conclusions on the predictive importance of this marker on the efficacy of erlotinib could not be made. Further studies are needed to clarify the markers that are predictive of erlotinib efficacy in Japanese patients, not only *EGFR* mutations, but also *KRAS* mutations and other as yet unidentified, biomarkers.

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Survival Prediction for Pancreatic Cancer Patients Receiving Gemcitabine Treatment*

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Although gemcitabine monotherapy is the standard treatment for advanced pancreatic cancer, patient outcome varies significantly, and a considerable number do not benefit adequately. We therefore searched for new biomarkers predictive of overall patient survival. Using LC-MS, we compared the base-line plasma proteome between 29 representative patients with advanced pancreatic cancer who died within 100 days and 31 patients who survived for more than 400 days after receiving at least two cycles of the same gemcitabine monotherapy. Identified biomarker candidates were then challenged in a larger cohort of 304 patients treated with the same protocol using reverse-phase protein microarray. Among a total of 45,277 peptide peaks, we identified 637 peaks whose intensities differed significantly between the two groups ($p < 0.001$, Welch's t test). Two MS peaks with the highest statistical significance ($p = 2.6 \times 10^{-4}$ and $p = 5.0 \times 10^{-4}$) were revealed to be derived from α_1-antitrypsin and α_1-antichymotrypsin, respectively. The levels of α_1-antitrypsin ($p = 8.9 \times 10^{-8}$) and α_1-antichymotrypsin ($p = 0.001$) were significantly correlated with the overall survival of the 304 patients. We selected α_1-antitrypsin ($p = 0.0001$), leukocyte count ($p = 0.066$), alkaline phosphatase ($p = 8.3 \times 10^{-8}$), and performance status ($p = 0.003$) using multivariate Cox regression analysis and constructed a scoring system (nomogram) that was able to identify a group of high risk patients having a short median survival time of 150 days (95% confidence interval, 123–187 days; $p = 2.0 \times 10^{-15}$, log rank test). The accuracy of this model for prognostication was internally validated and showed good calibration and discrimination with a bootstrap-corrected concordance index of 0.672. In conclusion, an increased level of α_1-antitrypsin is a biomarker that predicts short overall survival of patients with advanced pancreatic cancer receiving gemcitabine monotherapy. Although an external validation study will be necessary, the current model may be useful for identifying patients unsuitable for the standardized therapy. *Molecular & Cellular Proteomics 9:695–704, 2010.*

Invasive ductal adenocarcinoma of the pancreas is one of the most aggressive and lethal malignancies (1). It is the fifth leading cause of cancer-related death in Japan and the fourth in the United States, accounting for an estimated >23,000 deaths per year in Japan and >33,000 in the United States (2, 3). Because the majority of patients have distant metastases even at their first presentation (4, 5), the main therapeutic modality for pancreatic cancer is systemic chemotherapy, and gemcitabine monotherapy is the current standard (6). Gemcitabine treatment has significantly improved the median survival time of patients with advanced pancreatic cancer (7). However, the outcome of the treatment varies significantly among individuals, and a considerable portion of patients do not appear to benefit significantly from it. It therefore seems necessary to assess the efficacy and adverse effects of the drug before administration and tailor the treatment accordingly for each person.

We previously identified a predictive biomarker for hematologic toxicity, which is one of the most frequent and potentially life-threatening adverse effects associated with gemcitabine monotherapy (8). As a next step, we performed a large scale proteome analysis in this study to identify biomarkers predictive of patient survival after gemcitabine monotherapy. Several factors and their combinations have been reported to correlate significantly with outcome in patients with advanced pancreatic cancer receiving gemcitabine, such as performance status, metastases, serum albumin, alkaline phosphatase, and peripheral leukocyte count (9–11). Unfortunately, however, the accuracy of survival prediction based on these conventional prognostic factors seems unsatisfactory (9).

In recent years, there has been considerable interest in applying advanced proteomics technologies to the discovery of predictive biomarkers (12, 13). We and others have

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TABLE I

Clinical and laboratory data of patients with short term or long term survival

Survival time was calculated from the date of starting gemcitabine therapy until the date of death from cancer. Wilcoxon test was applied to assess differences in values. 5-FU, 5-fluorouracil; LAPC, locally advanced pancreatic cancer; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; C_{max}, peak concentration; AUC, area under the curve.

	Short term survivor (<100 days)	Long term survivor (>400 days)	*p*
No. of patients	29	31	
Sex (no. of patients)			0.361[a]
Male	21	19	
Female	8	12	
Age, mean (S.D.) (years)	63 (7)	67 (8)	0.123
ECOG performance status (no. of patients)			0.008[a]
0	8	20	
1	18	11	
2	3	0	
Body surface area, mean (S.D.) (m^2)	1.59 (0.17)	1.54 (0.15)	0.333
Prior therapy			0.438[a]
None	27	27	
Chemoradiotherapy using 5-FU for LAPC	2	4	
Clinical stage[b]			0.697[a]
IVa	2	3	
IVb	27	28	
Subsequent line chemotherapy after gemcitabine			0.045[a]
None	29	27	
Yes	0	4	
Leukocytes, mean (S.D.) ($\times 10^3/mm^3$)	7.6 (3.6)	5.2 (1.3)	0.002
Platelets, mean (S.D.) ($\times 10^4/mm^3$)	24.5 (7.6)	20.2 (4.6)	0.020
Hemoglobin, mean (S.D.) (g/dl)	11.7 (1.6)	11.7 (1.5)	0.491
Albumin, mean (S.D.) (g/dl)	3.4 (0.4)	3.7 (0.3)	0.014
Creatinine, mean (S.D.) (mg/dl)	0.70 (0.23)	0.68 (0.23)	0.726
AST, mean (S.D.) (IU/liter)	40 (25)	26 (15)	0.010
ALT, mean (S.D.) (IU/liter)	51 (44)	27 (19)	0.037
ALP, mean (S.D.) (units/liter)	728 (632)	337 (160)	0.026
Pharmacokinetic parameters of gemcitabine			
C_{max}, mean (S.D.) (μg/ml)	24.02 (7.52)	24.91 (6.22)	0.610
AUC, mean (S.D.) (h·μg/ml)	10.24 (2.83)	10.75 (2.32)	0.270
α_1-Antitrypsin,[c] mean (S.D.)	64.6 (66.8)	16.9 (7.9)	0.0003
α_1-Antichymotrypsin,[c] mean (S.D.)	706.4 (416.0)	389.0 (216.5)	0.0005
Tumor response[d]			<0.0001[a]
Complete response	0	0	
Partial response	0	1	
Stable disease	2	22	
Progressive disease	24	0	
Not evaluable	3	8	

[a] Calculated by χ^2 test.

[b] According to Ref. 23.

[c] Intensity of the corresponding peak measured by quantitative mass spectrometry.

[d] Evaluated after the first two cycles of gemcitabine monotherapy.

successfully applied MALDI MS-based protein profiling techniques for predicting the efficacy of chemoradiotherapy and molecular targeting therapy (14, 15). Two-dimensional image converted analysis of liquid chromatography and mass spectrometry (2DICAL)[1] is a new LC-MS-based proteomics platform that was developed in our laboratory (16). 2DICAL can quantify protein content accurately across a theoretically unlimited number of samples without isotope labeling and thus has considerable advantages over conventional LC-MS-based methods for clinical studies (17). The predictive biomarker protein for hematologic toxicity described above was identified using 2DICAL (8).

It has been generally accepted that tumor responses do not always correlate with the outcome of patients (10, 18, 19). The rates of complete and partial responses (Response Evaluation Criteria in Solid Tumors guideline) to gemcitabine mono-

[1] The abbreviations used are: 2DICAL, two-dimensional image converted analysis of liquid chromatography and mass spectrometry; AIC, Akaike's information criterion; CC, correlation coefficient; CI, confidence interval; CV, coefficient of variance; ECOG, Eastern Cooperative Oncology Group; NCC, National Cancer Center; ID, identification; FDR, false discovery rate.